The Patten reference is directed to "evolving" a protein encoded by a DNA substrate molecule by digesting the DNA from at least two molecules, generating a library of DNA molecules, screening the library for a desired property, and recovering the DNA molecule that encodes an evolved protein. This technique is referred to as "shuffling". More specifically, the Patten reference discloses methods of optimizing expression of a protein in a host cell (defined on column 9, lines 4-32 to include yeast) by providing a set of oligonucleotides, assembling the oligonucleotides into a library, expressing the library in a host cell, screening for improved expression of the protein and recovering a DNA substrate molecule encoding an evolved protein (column 3, lines 16-33). The Patten reference also discloses screening a library of mutants of a DNA substrate encoding a protein for an evolved DNA substrate by providing a library of mutants comprising an expression vector, transfecting a host cell with the library, wherein the mutant protein is expressed on the surface of the cell, screening with a ligand for the protein, recovering DNA encoding mutant protein and recovering an evolved DNA substrate (column 6, lines 16-30).

The Office Action stated the Patten reference "teaches mutating TCRs in order to improve the affinity of TCRs." The Office Action does not cite a specific portion of the Patten reference where this is shown. The Patten reference only mentions TCRs four times:

- paragraph bridging columns 26 and 27--T cell receptor is mentioned as a protein which is suited to the approach of evolving the chaperonin genes to possibly assist in preventing aggregation of folding intermediates (this reference is directed to mutating the chaperonin gene);
- column 42, lines 59-67--selection for chimeras that bind one receptor complex on monocytes but have reduced affinity for a high affinity complex on activated T cells (note this mention of T cells is not directed to mutating the T cell);
- column 52, lines 26-34--improvement of the affinity of T cell receptors for ligands of interest (i.e. MHC/tumor peptide antigen complexes) is listed as a "typical example" of the use of the method disclosed; and
- column 53, lines 8-13--in a description of mutating human interferon-alpha genes (IFN-α's), activation of cytotoxic T cells is mentioned as one biological effects of IFN-α's (note this

mention of T cells is not directed to mutating the T cell).

There are no examples in Patten showing the use of TCRs or the techniques used to mutate TCRs to improve the affinity of the TCRs. The only relevant mention of T cells in Patten is column 52, lines 26-34. Patten does not provide a working example or any description of how to produce high affinity TCRs. In fact, prior to the present invention, all attempts to produce high affinity TCRs failed. The references attached as Exhibit A and B refer to high affinity TCRs of the current inventors, but notably not to other researchers (see reference 12 in Malissen, B. "Les liaisons dangereuses" Nature Immunology 2(3)(2001) p. 196-198 and reference 5 of Foote, J. et al. "Breaking the Affinity Ceiling for Antibodies and T cell Receptors" PNAS 97(20) (2000) p. 10679-10681).

The Office Action stated the Dau reference "recites mutant TCRs having one or more mutations in the CDR regions (CDR3α and CDR3β) wherein the ligand of said TCR is a peptide/MHC ligand and methods of identifying said mutants." The Office Action also states Dau et al. teaches that "mutations in the CDR3α and CDR3β are important in ligand binding specificity."

Dau discloses a method for analyzing the gene fragment length and sequence of nucleotides that encode the CDR3α and β regions of the T cell receptor by obtaining cells, generating an assay gene fragment length profile, and comparing the assay profile to a control profile from a healthy subject. A T cell immunoproliferative condition is correlated with a gene fragment length found to a greater extent in the assay profile than in the control profile.

Example 7 of Dau (referred to in the Office Action) describes comparison of the gene fragment length from a patient having cirrhosis with healthy subjects. The analysis indicated some fragments that were present in sick subjects were not present in the healthy subject.

It is not seen where Dau teaches "mutant TCRs having one or more mutations in the CDR regions (CDR3α and CDR3β) wherein the ligand of said TCR is a peptide/MHC ligand" as stated by the Office Action. The only references to MHC in the Dau reference are in column 1,

lines 41-67, where Dau reports that "since both α and β chain CDR3 lengths are consistent with directly contacting peptide antigens, they have been postulated to be constrained in size because of their evolutionary selection for binding to peptide-MHC complexes."

It is not seen where Dau teaches "that mutations in the CDR3α and CDR3β are important in ligand binding specificity" as stated by the Office Action.

Dau does not teach anything about the affinity of TCRs for ligands or the increase of such affinity. Specificity and affinity of antigen recognition are different concepts. Dau (and the other references cited) do not address questions such as: why are the affinities of natural TCRs always very low? can the affinity of a TCR be increased, especially given that conformational mobility of TCRs could be necessary for specificity? and if the affinity of a TCR can be increased, what impact would it have on specificity? The answers to these questions were simply not available until the present invention. Prior to the present invention, it was reasonable to believe that an increase in affinity would lead to TCRs that cross-reacted with every peptide bound to the same MHC. This possibility stems from the differences in antigens recognized by antibodies and TCRs. With antibodies, where higher affinities have been engineered many times, the antigen is well defined and the epitope is typically unique. In contrast, the MHC portion of the antigenic epitope for TCRs will be shared among all antigenic peptides bound to this MHC. Thus, it is easy to imagine that manipulations of the TCR could lead to extensive high-affinity cross-reactivity. In fact, the crystal structures of several TCR:pepMHC complexes have shown that all six CDRs can contact the MHC (Garcia et al., "An αβ T cell receptor structure at 2.5 angstrom and its orientation in the TCR-MHC complex" Science (1996) 274: 209-219—cited in Information Disclosure Statement received by TC 1600/2900 October 31, 2001; Rudolph and Wilson, "The specificity of TCR/pMHC interaction" Curr. Opin. Immunol. (2002) 14: 52-65 attached as Exhibit C). The possibility that all six CDRs can interact with the MHC helices is not what was predicted by many, including Dau *et al.* The present invention shows for the first time that it is possible to generate high-affinity TCRs, and yet they also retain peptide specificity.

The Office Action stated the Reinherz reference "teaches the generation of soluble TCRs

and the desirability of generating soluble TCRs as probes to identify antigen/MHC complexes in vivo, including those responsible for autoimmune diseases."

Reinherz does not provide any example or direction on how to bind specific antigen/MHC complexes. The soluble T cell receptor developed by Reinherz would not work for this purpose because the affinity of the natural TCR for antigen/MHC complexes was much too low, and there is no known experiment using soluble scTCRs to detect antigen/MHC on the surface of cells.

From the references individually and in combination, the invention as a whole is nonobvious. All limitations of claim 44 are not shown in the combination of references. Claim 44 recites a soluble mutant TCR with high affinity for a ligand and one or more mutations in a CDR. No TCRs in the references cited have high affinity for a ligand. Prior to the invention, there was no method to make the TCR as claimed in claim 44. The invention as claimed in claim 44 has advantages, properties, utilities and unexpected results over those TCRs developed before, and the solution to the problem of making high affinity TCRs with one or more mutations in a CDR was not apparent until the claimed invention was made.

There is no suggestion in the references themselves to combine the teachings or make any modifications. There is no suggestion in Patten regarding the desirability of mutating the CDR regions of the TCR or making soluble TCRs. There is no suggestion in Dau regarding the desirability of increasing the affinity of TCR for a ligand, and no suggestion in Dau of using TCRs to bind to the peptide/MHC ligand, since Dau is concerned with comparing the fragment length of patients having disease and healthy patients. There is no suggestion in Reinherz regarding increasing the affinity of TCR for a ligand.

Also, there is no reasonable expectation for success if the references were combined. The soluble TCR used in Reinherz would not bind with high affinity to a ligand, and there is no suggestion in any of the references regarding the desirability of modifying the reference to produce a TCR that binds with high affinity to a ligand, and certainly no direction to do so.

The Office Action states that

> the ordinary skilled artisan, seeking to generate soluble TCRs having higher affinity for a ligand and having one or more mutations in the CDR3α-β, would have been motivated to combine the teachings of Patten et al. on the generation of mutant TCRs so as to improve the affinity of said TCRs for ligands of interest with the teachings of Dau et al. on the importance of mutations in the CDR3α-β regions of the TCRs in regulating binding of the TCR to the ligand (which is usually a peptide/MHC ligand) and the teachings of Reinherz et al. on the desirability of generating soluble TCRs as therapeutic agents for the treatment of autoimmune diseases in vivo in order to mutate TCRs in a region known to be involved in ligand binding (the CDR3α-β regions) and render them soluble for the desired effect of using them as therapeutic agents for treatment of autoimmune diseases wherein the mutated TCRs bind more effectively to the antigen/MHC complexes on antigen presenting cells in vivo and hence prevent activation of autoreactive T cell clones (as taught by Reinherz et al.).

Certainly this is a desirable outcome. However, no one before the present inventors had been able to succeed in achieving this outcome. The teachings of the cited references do not provide any guidance in achieving this goal, they merely recite certain aspects of the overall goal that would be desirable. The inventors of the present invention showed that they could engineer higher-affinity TCRs, that these TCRs were indeed antigen/MHC-specific, and that soluble forms of the high-affinity TCRs could detect antigen/MHC that was expressed on the surface of cells *in vivo* for the first time.

In view of the above arguments, it is believed that claims 44-46 are allowable. Reconsideration and withdrawal of the rejection is respectfully requested. New claims 104-106 have been added. These claims are identical to claims 44-46 with the addition of a dissociation constant. It is believed new claims 104-106 are also allowable.

112, second paragraph rejection

Claims 38-57 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite.

Claim 44 (and dependent claims) was rejected as allegedly vague in the recitation of the phrase "having high affinity" for a ligand. The Office Action states no definition is found in the specification for this phrase. The phrase "having high affinity" for a ligand is defined in the

specification on page 4, lines 10-14 as "higher affinity than wild type for the cognate ligand" and on page 4, lines 12-14 as desirably from about 10^7 to about 10^{10}. The affinity of wild type TCR for ligands is known (or easily determined by one of ordinary skill in the art). Therefore, a TCR having higher affinity than wild type is also easily determined. In view of the above argument, it is believed that claim 44 (and dependent claims) is not indefinite. Reconsideration and withdrawal of the rejection is respectfully requested.

The Office Action rejected claims 38, 39, 48 and 49 (and dependent claims) as allegedly vague in that no units of measure are associated with the dissociation constants. The units of the dissociation constants are known in the art (for example, Eisen, et al. (1996) "Antigen-specific T-cell receptors and their reactions with complexes formed by peptides with major histocompatibility complex proteins," Adv. Protein Chem. 49:1-56; and Holler et al. (2000) "In vitro evolution of a T cell receptor with high affinity for peptide/MHC," PNAS 97:5387-5392, both cited in the Information Disclosure Statement filed July 31, 2001) and are M^{-1}. The Eisen reference was cited in the specification as filed on page 14, line 2 and was incorporated by reference on page 34, lines 24-25. Also, units of dissociation constants are present in the specification as filed, on page 13, line 30 to page 14, line 1, for example. For clarification, claims 38, 39, 48 and 49 have been amended to include the unit M^{-1}. No new matter is added by this amendment.

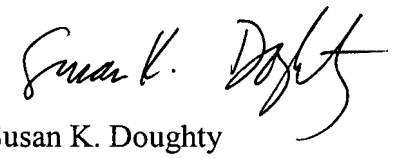
The Office Action rejected claim 54 (and dependent claims) as allegedly vague in the recitation of the term "mutant high affinity TCR". The Office Action stated it was unclear if the engineered TCR has been mutagenized in an additional fashion. For clarity, the word "mutant" in claim 54 has been removed. No new matter is added by this amendment.

CONCLUSION

In view of the above arguments and amendments, it is believed that claims 38-57 and 104-106 are allowable. Reconsideration and withdrawal of the rejections is respectfully requested. If there are any issues remaining to passage of the case to issuance, the Examiner is respectfully requested to telephone the undersigned.

This response is accompanied by a check in the amount of $120.00 for one independent claim and two dependent claims. It is believed that the current submission does not require the payment of any other fees. If this is incorrect, however, please credit any overpayment or charge any requisite fees to Deposit Account No. 07-1969.

Respectfully submitted,



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